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Short communication

A robust and easily reproducible protocol for the determination of size and size distribution of iron sucrose using dynamic light scattering

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ABSTRACT

Iron sucrose (IS), a nanocolloidal solution used in the treatment of iron deficiency anemia, is currently under investigation for the elucidation of its critical quality attributes. Assessment of IS's size and size distribution has been recently attempted using dynamic light scattering (DLS). However, due to heterogeneous interpretation of DLS data, variable results were retrieved. The aim of this work was to establish a simple and reproducible DLS protocol to unequivocally define the size and size distribution of IS by using size distribution approximation in Number. Underlining the limitations of the commonly used DLS approximations, we identified the drug as being composed of a population of monodisperse nanoparticles of about 7 nm in diameter. The method here described might therefore be useful for the evaluation of quality, safety and efficacy of IS and its follow-on versions.

1. Introduction

Iron sucrose (IS) is an injectable colloidal solution composed of iron(III)-oxyhydroxyde nanoparticles coated and stabilized with polymeric sucrose [1,2]. IS is widely employed to rapidly relieve severely iron deficient patients either not responding to or not tolerating oral iron supplementation [3]. Moreover, this synthetic medicine belongs to the group of non-biological complex drugs (NBCDs), a pharmaceutical class of drugs with a degree of complexity comparable to or possibly higher than biologicals [4–6]. With the lack of a specific regulatory pathway for follow-on NBCD products, the "generic" paradigm has in the past been adopted for their marketing authorization [7–9]. Nevertheless, several studies reported that the use of either IS or a follow-on IS product did not induce the same clinical outcomes, underlining the inadequacy of the generic approach for these drugs [10–16]. Quality, safety and efficacy of IS products are strictly related to their complex manufacturing process, which might engender the presence of residual sucrose as well as of free iron in association with the nanoparticles [17–19]. Minor modifications of the process variables (environmental conditions, formulation parameters and preparation, equipment utilized) can lead to significant changes in structure, stability and performances of the final product [20–22]. New specific assays to determine the physicochemical properties of these complex nanomedicines are therefore essential to assess drug sameness as well as to predict their behavior in the biological environment [23–25]. Despite the growing interest of regulatory authorities and the scientific community in the topic [26–29], most of IS's physicochemical characteristics remain undefined and the identification of its critical quality attributes is still uncertain. Recently, lists of suitable assays for the physicochemical characterization of IS have been released, indicating dynamic light scattering (DLS) as a procedure to evaluate size and size distribution [30,31]. Over the last few years several authors have already established DLS protocols to elucidate IS nanoparticle dimensions [32–34]. With the procedure settings being slightly different and in the absence of a standardized method to interpret the results, ambiguous information was obtained on size and size distribution of IS. The diverse approaches for data reporting might limit the correct evaluation of these characteristics for comparison purposes also in association with other orthogonal methods [35,36]. Moreover, the protocols publically available for IS did not investigate the hypothetical influence of the collective diffusion of sucrose in the suspension related to the presence of free polymer in the formulation [30,37]. The accurate elucidation of IS's size and size distribution is therefore compulsory. Slight variations in these parameters have been related to pivotal changes of IS's pharmacokinetics as well as its biodistribution and therapeutic profile [38,39]. In the framework of the revision of a new monograph in the European Pharmacopoeia (Ph. Eur.) on iron sucrose concentrated solution, we developed a robust and easily repro-

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ducible protocol to clearly define size and size distribution of IS using DLS.

2. Materials and methods

Commercial samples of IS [Venofer®, 20 mg Fe/mL, batches 605211 (exp. 10.2019), 678701 (exp. 07.2019) and 693201YA (exp. 09.2019)] were kindly provided by Vifor Pharma LTD. (Switzerland). According to the method previously described by Jahn et al. [34], the colloidal solutions were diluted using a dilution factor of 50 x with ultrapure water to the final concentration of 0.4 mg Fe/mL (n = 6). The use of traces of ionic additive was avoided to prevent possible sample alteration, as allowed for specific cases by the guideline ISO 22412:2017 [40]. Particle size and size distribution were determined using a Zetasizer Nano ZS (Malvern, UK) at a scattering angle of 173° and a He-Ne laser beam at λ = 633 nm. Refractive index was set at 1.334 [41] and absorbance was considered to be equal to 0.300. All measurements were carried out at 25.0 ± 0.2 °C in disposable polystyrene cuvettes. The equilibration time was set at 60 s. Each result is the average of three sub runs constituted by at least ten measurements.

3. Results

The mean hydrodynamic diameters and standard deviations (SD) of Z-Average, polydispersity index (PDI), size distribution in Volume and size distribution in Number as well as their relative standard deviations (RSD) for the three IS batches investigated (n = 6) are summarized in Table 1.

4. Discussion

DLS is a routine technique used to determine size and size distribution of nanoparticles in colloidal suspensions [42]. The modest price of the equipment, the easiness of use together with the possibility of extensive data analysis made DLS the most common method to evaluate nanomedicine particle characteristics [36].

DLS is equipped with software (Malvern Zetasizer® software v. 7.11) that provides three different alternatives to quantify the size distribution based on Intensity, Volume or Number. In addition, data are often reported as Z-average and Polydispersity Index (PDI), which are derived from the Intensity weighted correlation function using the "cumulant method" analysis [43]. Despite the comprehensive use, Intensity based results may present some inaccuracies. In fact, the presence of aggregates or large particles might dramatically affect Z-average and PDI leading to incorrect results [44,45]. Few big particles in the sample bulk will influence the signal, provoking an overestimation of the specimen's mean hydrodynamic diameter [46]. A more detailed discussion on DLS principles is found in a recent review by Bhattacharjee [47]. When analyzing the three IS batches, Z-average was measured to be approximately 12 nm with a variable RSD of maximum 4.1%, suggesting good repeatability of the assay. Moreover, PDI values indicated a broad size distribution of nanoparticles at moderate polydispersity. As visible in Fig. 1A, the size distribution by Intensity chart for IS batch 605211 displays a bimodal distribution of particles. However, this second peak becomes insignificant when switching from Intensity to either Volume (Fig. 1B), or Number size distribution (Fig. 1C).

In addition, the United States Pharmacopeia (USP) Convention states that IS must contain between 260 and 340 mg of sucrose/mL drug product, with an average iron:carbohydrate ratio of 1:15 [48]. Given the complex synthetic pathway of IS, the large excess of carbohydrate is most likely correlated to a fraction of loosely bound sucrose in the final IS formulation [17]. Moreover, it has been previously reported that concentrated solutions of sucrose present a

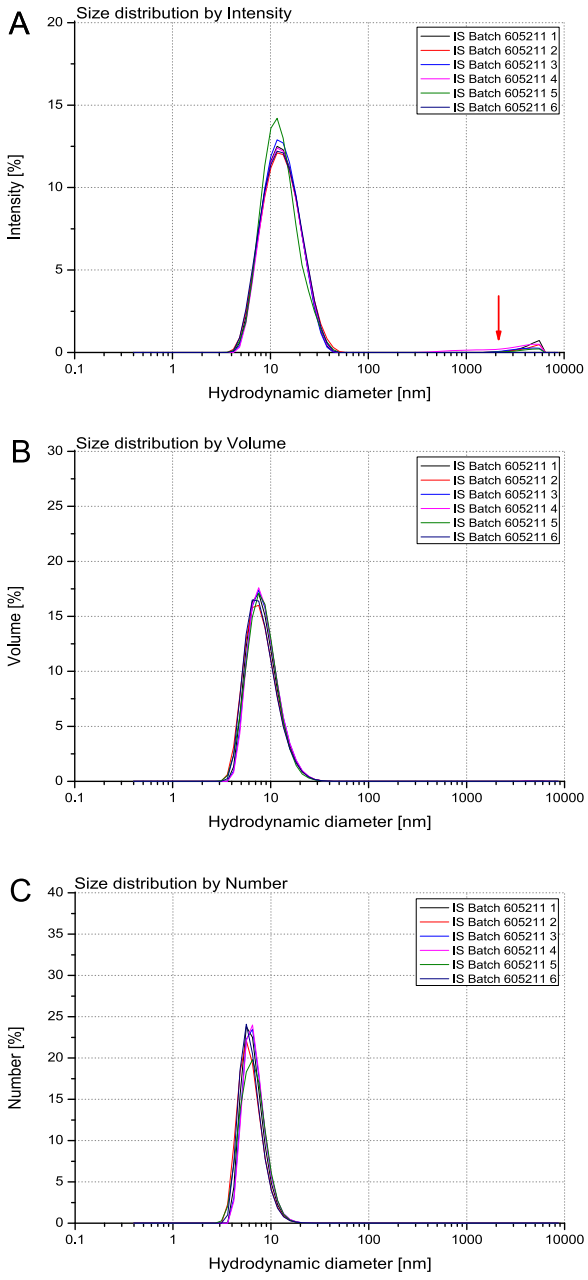

Fig. 1. Size distribution by Intensity (A), Volume (B) and Number (C) for six independently prepared samples of IS batch 605211 at the concentration of 0.4 mg Fe/mL in ultrapure water. For image resolution purposes, raw data from Malvern Zetasizer® software v. 7.11 were plotted with Origin Pro® v.8.5 with no further modification. Charts of IS batch 678701 and IS batch 693201YA showed the same trend.

secondary bump exclusively when using the size distribution in Intensity between 100 nm and 1 μm due to the collective diffusion of the sucrose polymer [37,49].

In order to ascribe the bump visible for IS in the Intensity size distribution to either a second population of nanoparticles or to the presence of "contaminants" in the sample, the evaluation of the size and size distribution for an aqueous solution of sucrose Ph. Eur. at the concentration of 6 mg/mL was carried out (n = 6). The concentration chosen for the assay is theoretically comparable to the amount of sucrose in the IS bulk after a dilution of 50 x.

The size distributions in Intensity, Volume and Number for the sucrose solutions are reported in Fig. 2(A–C). The size distribution in Intensity of the sucrose solution presents a multimodal distribution with several bumps in the range 100 nm–1 μm. These peaks disap-